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AUTHOR(S):

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# Advanced Research Center for Beam Science – Structural Molecular Biology –

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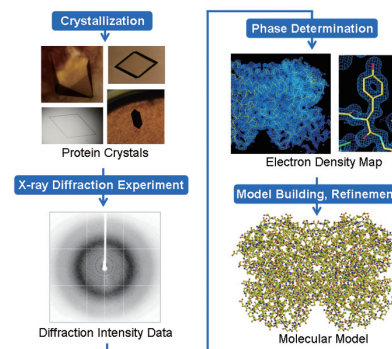
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## Scope of Research

The research activities in this laboratory are performed for X-ray structural analyses of biological macromolecules and the investigation of the electronic state in materials as follows: The main subjects of the biomacromolecular crystallography are crystallographic studies on the reaction mechanism of enzymes, the relationship between the multiform conformation and the functional variety of proteins, and the mechanism of thermostabilization of proteins. In the investigation of the chemical state in materials, the characteristics of the chemical bonding in the atom and molecules are investigated in detail using a newly developed X-ray spectrometer with a high-resolution in order to elucidate the property of materials. The theoretical analysis of the electronic states with DV- $X\alpha$  and WIEN2k, and the development of new typed X-ray spectrometer with ultra high-resolution have also been carried out.

### KEYWORDS

Crystal  
X-ray Crystallographic Analysis  
Racemase  
Thermostability  
D-Amino Acid



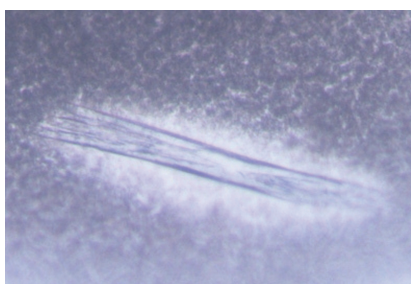
## Selected Publications

- Fujii, T.; Maeda, M.; Mihara, H.; Kurihara, T.; Esaki, N.; Hata, Y., Structure of a NifS Homologue: X-ray Structure Analysis of CsdB, an *Escherichia coli* Counterpart of Mammalian Selenocysteine Lyase, *Biochemistry*, **39**, 1263-1273 (2000).
- Fujii, T.; Sakai, H.; Kawata, Y.; Hata, Y., Crystal Structure of Thermostable Aspartase from *Bacillus* sp. YM55-1: Structure-based Exploration of Functional Sites in the Aspartase Family, *J. Mol. Biol.*, **328**, 635-654 (2003).
- Hayashida, M.; Fujii, T.; Hamasu, M.; Ishiguro, M.; Hata, Y., Similarity between Protein-Protein and Protein-Carbohydrate Interactions, Revealed by Two Crystal Structures of Lectins from the Roots of Pokeweed, *J. Mol. Biol.*, **334**, 551-565 (2003).
- Fujii, T.; Oikawa, T.; Muraoka, I.; Soda, K.; Hata, Y., Crystallization and Preliminary X-ray Diffraction Studies of Tetrameric Malate Dehydrogenase from the Novel Antarctic psychrophile *Flavobacterium frigidimaris* KUC-1, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **63**, 983-986 (2007).
- Fujii, T.; Goda, Y.; Yoshida, M.; Oikawa, T.; Hata, Y., Crystallization and Preliminary X-ray Diffraction Studies of Maleylacetate Reductase from *Rhizobium* sp. Strain MTP-10005, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **64**, 737-739 (2008).

## Crystallographic Structure Analysis of Aspartate Racemase from *Lactobacillus sakei* NBRC-15893

Amino-acid racemases are responsible for the racemization of amino acids and can be mainly grouped into two families, pyridoxal 5'-phosphate (PLP) dependent and PLP independent. Aspartate racemase catalyzes the inter-conversion between L- and D-aspartate and belongs to the PLP-independent racemase group. The enzyme is thought to employ a two-base mechanism to catalyze both the directions of racemization and utilize two cysteine residues as the conjugated catalytic acid and base in the catalytic reaction. Only the crystal structure of aspartate racemase from a hyperthermophilic archaeon has been reported so far. To elucidate the structure-function relationship of aspartate racemase which works in the range of the low to medium temperature, we have determined the crystal structure of aspartate racemase from *Lactobacillus sakei* NBRC-15893 (LsAspR).

Initial crystallization experiments were performed by the sitting-drop vapour-diffusion method using Crystal Screen I, Crystal Screen II, and PEGRx. Small crystals were obtained after several days with solution PEGRx #58. The crystallization conditions were optimized based on those of the solution. The final conditions produced rod-shaped crystals with approximate dimensions of  $0.4 \times 0.15 \times 0.03$  mm at 293 K in 3 days using the sitting-drop vapour-diffusion method with seeding technique (Figure 1). Drops of 1  $\mu$ l protein solution at 20 mg/ml (in 50 mM MES buffer, pH 6.5, 4 mM dithiothreitol) and 1  $\mu$ l reservoir solution were equilibrated against 100  $\mu$ l reservoir solution consisting of 25% (v/v) PEG-MME 550, 5% (v/v) 2-Propanol, and 0.1 M Sodium acetate, pH 4.8.

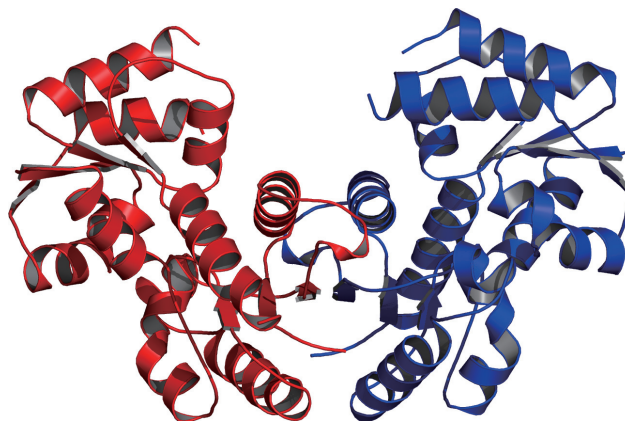


**Figure 1.** Crystal of aspartate racemase from *Lactobacillus sakei* strain NBRC-15893.

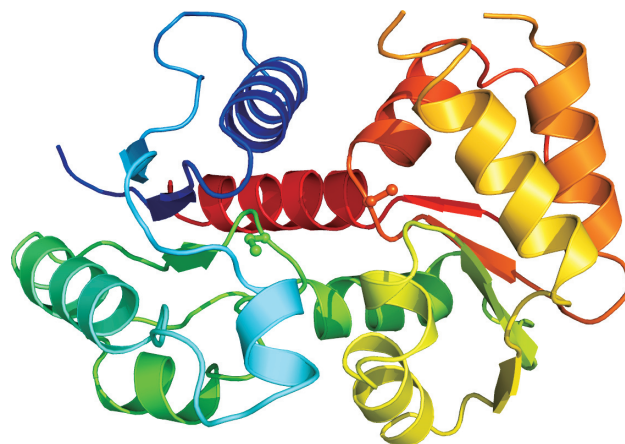
Diffraction experiments were performed at beamline NE-3A, Photon Factory AR, Tsukuba, Japan. The crystal was flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected at a wavelength of 1.000 Å using a Quantum 270 CCD detector set to 331.2 mm in a crystal-

to-detector distance. The crystals belonged to space group  $P3_121$  with unit cell parameters of  $a=b=105.5$  Å and  $c=96.5$  Å. The data set was collected at 2.6 Å resolution and has 20,826 independent reflections with completeness of 99.7%. The asymmetric unit contained one dimeric molecule of LsAspR with a corresponding crystal volume per protein mass ( $V_M$ ) of  $2.97$  Å<sup>3</sup>/Da and a solvent content of 59%. The crystal structure has been determined by molecular replacement. The current model was refined at 2.6 Å resolution to an  $R$ -factor of 23.8% ( $R_{\text{free}}=31.6\%$ ).

In crystals, LsAspR adopts a homodimeric form (Figure 2). The subunit consists of two domains: the N-terminal domain (residues 1–104 and 216–234) and the C-terminal domain (residues 105–215). In each domain, a central four-stranded parallel  $\beta$ -sheet is flanked by six  $\alpha$ -helices. The spatial arrangement of the strictly conserved residues Cys84 and Cys196 strongly indicates that the active site of LsAspR must be located in the cleft between the two domains (Figure 3).



**Figure 2.** Dimeric molecular structure of aspartate racemase from *Lactobacillus sakei* strain NBRC-15893.



**Figure 3.** Subunit structure of aspartate racemase from *Lactobacillus sakei* strain NBRC-15893. The strictly conserved residues Cys84 and Cys196 are shown as ball-and-stick models.